

FILE 'BIOSIS, MEDLINE, EMBASE, EMBAL, SCISEARCH, BIOTECHDS, CAPLUS'
ENTERED AT 16:20:27 ON 05 AUG 2003

L1 89876 S (EBV OR (EPSTEIN (1W) BARR (1W) VIRUS?))

L2 3957 S L1 AND VECTOR?

L3 436 S L2 AND (PCR OR (AMPLIF?))

L4 75 S L3 AND (PRIMER?)

L5 56 DUP REM L4 (19 DUPLICATES REMOVED)

L6 5 S L5 AND PY<1993

L6 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI
on STN

ACCESSION NUMBER: 1992-03039 BIOTECHDS

TITLE: A general and fast method to generate multiple site directed
mutations;
site-directed mutagenesis method using the polymerase
chain reaction

AUTHOR: Mikaelian I; *Sergeant A

LOCATION: ENS-CNRS UMR49, Ecole Normale Supérieure de Lyon, 46 Allée
d'Italie, 69364 Lyon Cedex 07, France.

SOURCE: Nucleic Acids Res.; (1992) 20, 2, 376

CODEN: NARHAD

DOCUMENT TYPE: Journal

LANGUAGE: English

SO Nucleic Acids Res.; (1992) 20, 2, 376

CODEN: NARHAD

AB. . . method to generate multiple site-directed mutations (deletion,
insertion or substitution) in a given DNA fragment. The method required
3 universal primers for the vector, and only 1
specific primer for each mutation. The method consisted of 2
successive rounds of polymerase chain reaction (PCR). The 1st
round consisted of 2 simultaneous PCRs, with primers (1 and 2)
homologous to the vector, but with a mismatched 3'-end in
primer 2. The 2nd PCR was done with primer 3
and primer M (with a mutation). Amplified fragments
were purified, mixed and subjected to another PCR round with
external primers 1 and 3 (with no amplification of
parental and hybrid B fragments). Only hybrid A was amplified,
and since the 3'-end of primer 2 was not complementary to the
DNA, only the mutated strand was amplified. The
amplified fragment could then be cleaved and ligated into a
vector. The method was used to generate 10 different deletion
and substitution mutants in Epstein-Barr
virus transcription factor EB1 or Z, with a routine efficiency of
90%. (3 ref)

| L Number | Hits | Search Text | DB | Time stamp |
|----------|------|---|---|------------------|
| 1 | 424 | (EBV (epstein adj1 barr adj1 virus)) SAME (detection) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/05 17:02 |
| 2 | 217 | ((EBV (epstein adj1 barr adj1 virus)) SAME (detection)) and (vector) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/05 17:02 |
| 3 | 190 | ((EBV (epstein adj1 barr adj1 virus)) SAME (detection)) and (vector)) and (pcr or amplifi\$7) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/05 17:02 |
| 4 | 154 | ((EBV (epstein adj1 barr adj1 virus)) SAME (detection)) and (vector)) and (pcr or amplifi\$7)) and primer | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/05 17:03 |
| 5 | 12 | ((EBV (epstein adj1 barr adj1 virus)) SAME (detection)) and (vector)) and (pcr or amplifi\$7)) and primer) and (EBV (epstein adj1 barr adj1 virus)).ti. | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/05 17:03 |

| L Number | Hits | Search Text | DB | Time stamp |
|----------|------|---|---|------------------|
| 1 | 12 | ((EBV (epstein adj1 barr adj1 virus)) and ((vca adj1 p18) (vca adj1 p40))) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/04 15:42 |
| 2 | 10 | ((EBV (epstein adj1 barr adj1 virus)) and ((vca adj1 p18) (vca adj1 p40))) and (bfrf3 bdrf1) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/04 15:42 |
| 3 | 17 | ((EBV (epstein adj1 barr adj1 virus)) and (BFRF3 BdRF1)) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/04 15:42 |
| 4 | 7 | ((EBV (epstein adj1 barr adj1 virus)) and (BFRF3 BdRF1)) not (((EBV (epstein adj1 barr adj1 virus)) and ((vca adj1 p18) (vca adj1 p40))) and (bfrf3 bdrf1)) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2003/08/04 15:43 |